Dosage-Dependent Proteome Response of *Shewanella oneidensis* MR-1 to Chromate Insult

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OVERVIEW

- *Shewanella oneidensis* MR-1 (Figure 1) is a gram-negative, facultatively anaerobic bacterium originally isolated from a freshwater lake. (*Science* 240, 1988, 1319-1320)
- *S. oneidensis* MR-1 has the ability to reduce toxic metal ions [e.g., Cr(VI) and U(VI)] found in industrial and governmental waste sites.
- Cells were grown and exposed to three different metal concentrations in order to probe the dosage response of *S. oneidensis* MR-1 to Cr(VI) in the form of chromate.
- Protein fractions were digested with trypsin and analyzed with a multidimensional HPLC-NanoESI-MS/MS protocol.
- The goal of this work is to identify protein components of pathways/mechanisms responsible for both detoxification and reduction of chromate.

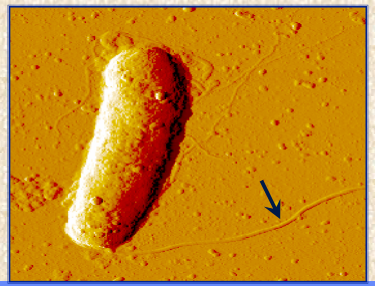


Figure 1: Atomic force microscopic image of *S. oneidensis* MR-1 grown under physiologically optimal conditions. The arrow points to the flagellum with other unknown appendages viewable around the cell. (Micrograph courtesy of K. Chourey)

EXPERIMENTAL

- ***S. oneidensis* MR-1 dosage response cells were grown under aerobic conditions with the addition of 0, 0.3, 0.5, or 1.0 mM K_2CrO_4 when cells reached mid-exponential phase. The cells were then allowed to grow for an additional 30 minutes in the presence of chromate.**
- **Cells were lysed using sonication and protein fractions were separated into crude and membrane fractions by centrifuging the samples at 100,000xg for 60 minutes. Following lysis, a trypsin digestion using a standard protocol was employed.**
- **Analysis was carried out by a 24 hour multidimensional HPLC-MS/MS protocol. (See Figure 2.)**
  - **Separation was accomplished by online 2-D chromatography using strong cation exchange as the first dimension and C18 reverse phase as the second dimension of separation.**
    - **A LTQ linear trapping quadrupole (Thermo Finnigan) and Ultimate HPLC (LC Packings) were operated in the data-dependent mode for the dosage response samples.**
- **Peptide identification was completed by the search engine SEQUEST with a two unique peptide cut-off (X-corr values 1.8(+1), 2.5(+2) and 3.5(+3)).**
- **Semi-quantitation: Proteins were considered differentially expressed (up- or down-regulated) with a difference in at least two of the following categories: 5 or more peptides, a difference of 40% sequence coverage, or 2X more spectra identified between treated and control samples.**

RESULTS

Global Results

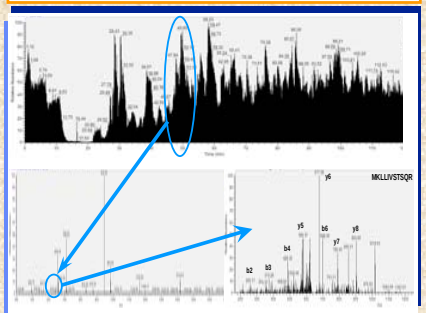
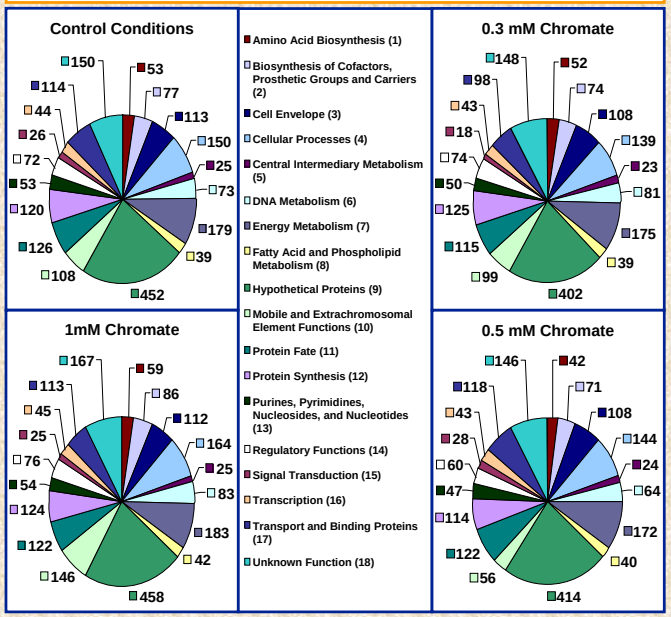


Figure 2: The ion chromatogram from the 0.3 mM chromate sample illustrates a full MS scan that contains a peak at m/z 638. This peak was subsequently isolated and fragmented giving the third spectrum, which contains the sequence of a peptide from SO3585 (see Figure 3) that was identified up-regulated at the 0.5 mM and 1.0 mM chromate levels.

Condition	Proteins Identified 1 pep*	Proteins Identified 2 pep#	Average Sequence Coverage$
Control	2593	1975	35.6%
0.3 mM	2419	1864	32.8%
0.5 mM	2393	1813	37.0%
1.0 mM	2630	2085	37.5%
Total non-redundant		2445	

*identified with at least 1 peptide per protein
#identified with at least 2 peptides per protein
$average sequence coverage at the 2-peptide level

Functional Category Assignments



Up-Regulated Proteins Under All Dosage Conditions

Locus	Control			0.3mM Chromate			0.5mM Chromate			1.0mM Chromate			Functional Category Number	Description
	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra		
SO0344	11.6%	3	3.5	31.5%	12	19	42.4%	20	30	48.2%	24.5	34	7	methylcitrate synthase (prpC)
SO1190	48.0%	11	19	55.3%	24	74	85.1%	32	102	70.2%	28	88	9	conserved hypothetical protein
SO1482	52.2%	26	35	76.40%	75	266	74.8%	81	328	70.5%	60	160	17	TonB-dependent receptor, putative
SO2426	0.0%	0	0	31.5%	7	10	22.4%	4	14	32.3%	8	13	15	DNA-binding response regulator
SO3030	4.0%	1	2	49.1%	24	37	47.7%	20	40	48.4%	22	39	17	siderophore biosynthesis protein (aleA)
SO3032	0.066	3.5	3.5	0.19	8	19	22.3%	10	22.5	0.28	15.5	25.5	17	siderophore biosynthesis protein, putative
SO3033	27.5%	15	23	60.40%	47	148	59.9%	45	172	57.0%	46	158	17	ferric alcaligin siderophore receptor
SO3420	54.4%	9	30	69.80%	16	58	75.8%	23	99	69.8%	20	66	7	cytochrome c
SO3462	5.8%	2	2	15.7%	7	10	27.5%	12	16	39.9%	15	23	6	DNA repair protein RecN (recN)
SO3667	25.2%	4	4	78.2%	21	78	91.4%	32	266	92.2%	34	241	9	conserved hypothetical protein
SO3669	11.2%	4	4	47.4%	30	205	74.8%	57	425	72.4%	54	199	17	heme transport protein (hugA)
SO3673	13.9%	3	3	40.9%	11	18	69.6%	22	74	69.1%	26	66	17	hemin ABC transporter, periplasmic hemin-binding protein (hmuT)
SO3675	0	0	0	0.271	5	10	40.9%	7.5	13	0.6455	10.5	27	17	hemin ABC transporter, ATP-binding protein (hmuV)
SO3738	6.6%	2	3	23.7%	8	18	20.4%	9	21	22.0%	8	19	5	sulfite reductase (NADPH) flavoprotein alpha-component (cysJ)
SO3914	0.445	29.5	49	0.668	66	236	65.5%	62	225.5	0.565	52.5	217	17	TonB-dependent receptor, putative
SO4743	67.1%	46	100	74.10%	70	307	75.0%	73	406	69.6%	61	266	17	TonB-dependent receptor, putative

Up-Regulated Proteins under Chromate Dosage Conditions

- **The dominant functional category was Transport and Binding Proteins (17) (9 proteins out of 16) for up-regulated proteins found under all dosage conditions. (See Figure 3.)**
  - **At the 1.0 mM Chromate level, 14 proteins up-regulated are annotated under this category.**
  - **For the 0.3 mM and 0.5 mM chromate exposed samples; 15 out of 42 and 18 out of 39 proteins were up-regulated and annotated as transport and binding proteins.**
- **Other dominant categories were Amino Acid Biosynthesis (1) and Energy Metabolism (7) at the 1.0 mM level with 7 and 12 proteins, respectively.**
  - **These categories contained between 1 and 3 proteins each for the 0.3 mM and 0.5 mM samples respectively; indicating a dosage response with respect to the 1.0 mM sample for these categories.**

Down-regulated Proteins Under All Dosage Conditions

Locus	Control			0.3mM Chromate			0.5mM Chromate			1.0mM Chromate			Functional Category Number	Description
	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra		
SO1776	40.0%	30.5	35.5	8.3%	3	3	23.8%	9	11	9.0%	4	6	3	outer membrane protein precursor MtrB (mtrB)
SO4053	38.4%	18	25.5	4.4%	2	2	15.5%	7.5	10	9.3%	6	19.5	4	methyl-accepting chemotaxis protein
SO0398	28.5%	13	18.5	3.7%	1.5	1.5	2.6%	1	1	6.1%	2	2	7	fumarate reductase flavoprotein subunit (frdA)
SO0847	47.4%	8	11	0.0%	0	0	8.6%	1	1	0.0%	0	0	7	iron-sulfur cluster-binding protein NapG (napG)
SO0848	66.4%	68	186.5	42.6%	35.5	52	67.2%	56	107.5	41.9%	34	60.5	7	periplasmic nitrate reductase (napA)
SO1429	20.4%	10.5	12.5	0.0%	0	0	7.9%	3.5	3.5	3.4%	1.5	1.5	7	anaerobic dimethyl sulfoxide reductase, A subunit (dmaA-1)
SO4513	42.3%	42	67.5	9.6%	7.5	9.5	23.3%	19	24.5	11.7%	8	11	7	formate dehydrogenase, alpha subunit
SO2175	33.4%	12.5	15.5	9.4%	3	3	25.8%	11	16	16.4%	5.5	7	9	conserved hypothetical protein
SO2929	69.6%	31	56	38.9%	14.5	25.5	38.3%	9.5	16.5	47.8%	14.5	21.5	9	hypothetical protein
SO0696	15.8%	10	15.5	2.5%	1.5	1.5	19.6%	9	11	6.8%	3	4	11	thiol:disulfide interchange protein DsbD (dsbD)
SO1805	23.4%	8	8	3.2%	1	1.5	22.0%	7.5	11	3.6%	1.5	1.5	17	peptide ABC transporter, periplasmic peptide-binding protein (sapA)
SO3865	37.1%	13	15.5	14.6%	2.5	3	30.8%	8	11	17.9%	4	5	17	molybdenum ABC transporter, ATP-binding protein (modC)
SO1674	42.5%	11	15	8.7%	1.5	2	36.5%	9	16	21.9%	4	4	18	oxidoreductase, short chain dehydrogenase/reductase family

Down-Regulated Proteins Under Chromate Dosage Conditions

- **The functional category of Energy Metabolism (7) demonstrated the greatest number of down-regulated proteins identified under all dosage conditions with 5 out of 13 proteins annotated under this category.**
  - **After exposure to 1.0 mM chromate; 9 proteins out of 26 were down-regulated and involved in energy metabolism.**
  - **After exposure to 0.3 mM and 0.5 mM chromate; 15 out of 86 and 9 out of 53 proteins were down-regulated and annotated as involved in energy metabolism.**
- **Other functional categories that had greater than 10 proteins down-regulated after exposure to 0.3 mM chromate were Cellular Processes (4) and hypothetical proteins (9).**
- **After exposure to 0.5 mM and 1.0 mM chromate; 8 and 9 proteins annotated as hypothetical proteins (9) were identified as down-regulated, respectively.**

CONCLUSIONS

- After exposure to three different concentrations of K_2CrO_4, we found:
  - **A total of 2,445 proteins identified under at least one of the four growth conditions.**
  - **128 proteins differentially expressed with respect to the control after the addition of 0.3 mM chromate (42 up- and 86 down-regulated).**
  - **96 proteins were differentially expressed after 0.5 mM chromate introduction (43 up- and 53 down-regulated).**
  - **A total of 92 proteins were differentially expressed after exposure to 1.0 mM chromate (66 up- and 26 down-regulated).**
- A total of 29 proteins were differentially expressed over all three chromate concentrations (See up- and down-regulated proteins tables).
- The 30-minute time point for the 1.0 mM chromate sample analyzed here is similar to our 45-minute 1.0 mM chromate shocked sample (Brown *et al*, MCP, in press).
  - **60% of proteins identified as differentially expressed after 45 minutes were also at the 30-minute time point.**
- A putative azoreductase (SO3585), a glyoxalase family protein (SO3586), and a hypothetical protein (SO3587) are identified only under chromate conditions (See Figure 2), and may be involved in a detoxification mechanism for chromate. Studies are underway to determine whether these proteins function in a complex and whether the putative azoreductase can reduce Cr(VI).
  - **SO3585 and SO3586 were found to be up-regulated at both the mRNA and protein level after 45 and 90 minutes of exposure to K_2CrO_4.**
  - **In this dosage response study, at a concentration of 0.3 mM chromate, SO3585 (3 peptides) and SO3586 (3 peptides) were identified at the protein level, but were not differentially expressed.**
  - **At the 0.5 mM chromate level, SO3585 was identified as up-regulated, however SO3586 and SO3587 were identified with 3 peptides each but not up-regulated.**
  - **At a concentration of 1.0 mM chromate only SO3585 is up-regulated with identification of SO3586 and SO3587 by 4 peptides each.**

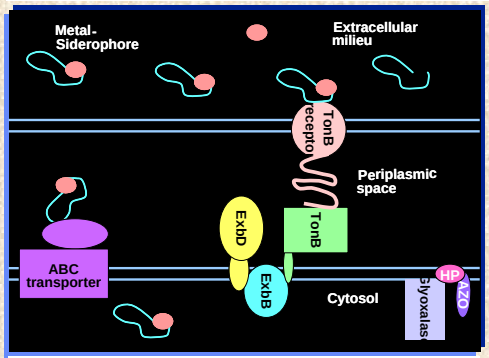


Figure 3: Schematic view of membrane region in Gram-negative bacterium. Key transport molecules are a metal-siderophore complex, TonB receptor, TonB complex (TonB, ExbB, & ExbD), and ABC transporter. These proteins are annotated under transport and binding proteins (17) in *S. oneidensis* MR-1 and are found up-regulated under the dosage response conditions presented here (See table of up-regulated proteins). Also shown is a putative azoreductase (AZO, HP, & Glyoxalase) complex of the inner membrane.

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